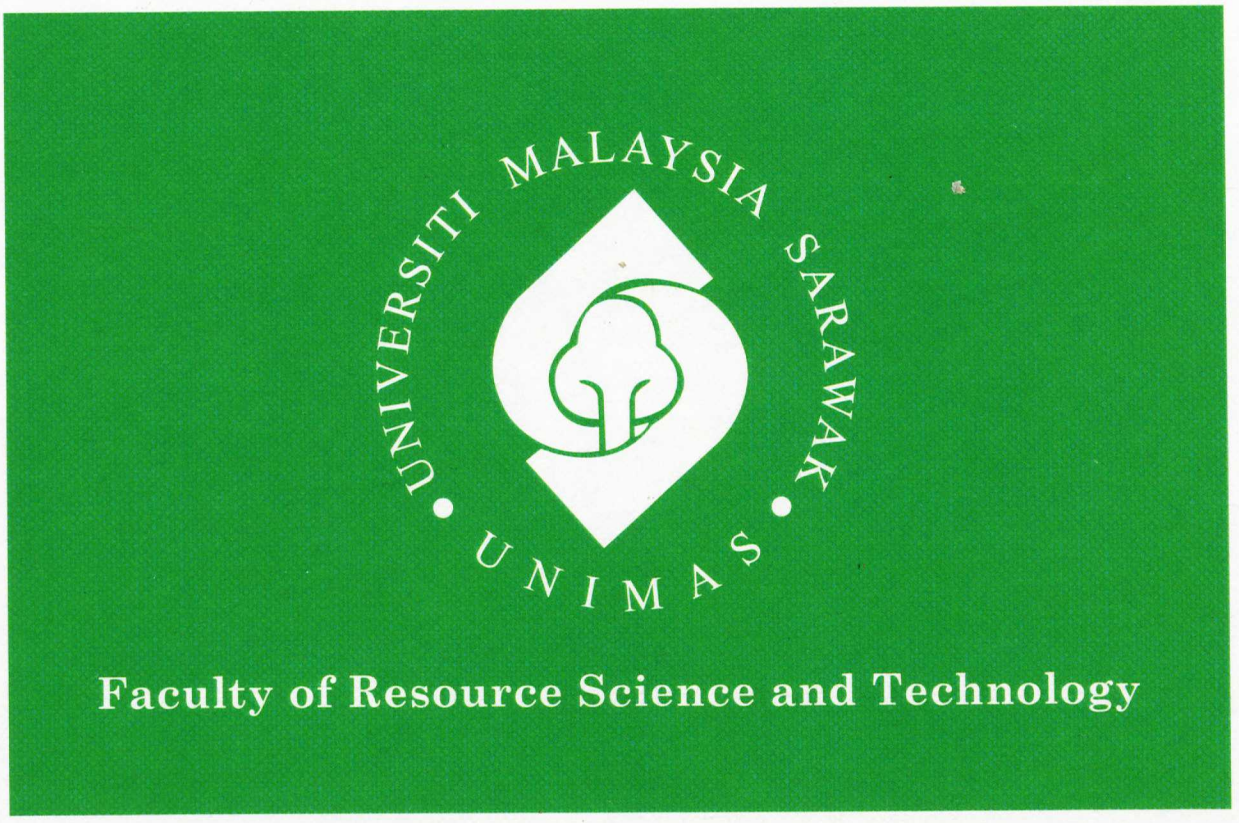

ANTIMICROBIAL ACTIVITY OF RED JERANGAU (*Boesenbergia Stenophylla*) EXTRACT AGAINST *E.coli, P. aeruginosa* AND *B. cereus*

Odealia Ak Likong
(50888)

Bachelor of Science with Honours
(Resource Biotechnology)
2018

ANTIMICROBIAL ACTIVITY OF RED JERANGAU (*Boesenbergia stenophylla*) EXTRACT AGAINST *E. coli, P. aeruginosa AND B. cereus*

ODEALIA AK LIKONG (50888)

The project submitted in partial fulfilment of requirement for the degree of Bachelor of Science with Honours (Resource Biotechnology)

Supervisor: Dr Samuel Lihan

WS47 Resource Biotechnology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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I, Samuel Lihan (SUPERVISOR'S NAME), hereby certify that the work entitled, Antimicrobial activity of red jerangau (Boesenbergia stenophylla) extract against E.coli, P. aeruginosa and B. cereus (TITLE) was prepared by the aforementioned or above mentioned student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment of Resource Biotechnology (PLEASE INDICATE THE DEGREE TITLE), and the aforementioned work, to the best of my knowledge, is the said student's work

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Thank you

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List of Abbreviations

TLC	Thin layer chromatography
Spp	Species
MHB	Mueller- Hilton Broth
MHA	Mueller- Hilton Agar
UV	Ultraviolet
ft	feet
%	Percentage
°C	Degree Celsius
rpm	Rotation per minute
(v/v%)	Volume / volume percentage
µg / µL	Microgram per microliter
µL	Microliter
h	Hour
RBC	Red Blood Cell
ETEC	Enterotoxigenic
EIEC	Enteroinvasive
EPEC	Enteropathogenic
PBS	Phosphate buffered saline
SDS	Sodium Dodecyl Sulfate
OD	Optical density
ddH2O	distilled water
Rf	Retardation factor
MIC	Minimum inhibitory concentration

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ABSTRACT

Boesenbergia stenophylla, otherwise known as Jerangau merah in Malay is an extremely rare species of medicinal plant belonging to the *Zingiberaceae* family. It can be found in high altitude place and it is endemic to Borneo. It has been claimed to have medicinal properties against bacteria which cause food poisoning. The local usually use it to cure stomach-ache, food poisoning and alcohol intoxication. This study was conducted in order to determine the antimicrobial activity of *B. stenophylla* by using agar well diffusion and minimum inhibitory concentration (MIC) against *Escherichia coli* (*E. coli)* strains EDL 993 and ATCC 25922, *Pseudomonas aeruginosa* (*P. aeruginosa*) strains ATCC 27853 and *Bacillus cereus* (*B. cereus*) strains ATCC 33019. A high antimicrobial activity was shown against *P. aeruginosa* in both agar well diffusion and minimum inhibitory concentration assay while the lowest antimicrobial activity against *B. cereus*. On the other hand, extracts that used hexane and ethyl acetate as a solvent system show high antimicrobial activity compared to the extract using water as a solvent system. In addition, extraction from stem shows more antimicrobial activity compared to rhizome and leaf. The extracts from *B. stenophylla* have shown antimicrobial activity and further studies should be carried out *in vivo* on the medicinal properties of the plant.

Key word: *B. stenophylla*, antimicrobial, agar well diffusion, minimum inhibition concentration, thin layer chromatography

Antimicrobial Activity of Red Jerangau (Boesenbergia stenophylla) against *E. coli, P. aeruginosa* and *B. cereus.*

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ABSTRAK

Boesenbergia stenophylla, yang dikenali sebagai Jerangau merah dalam bahasa Melayu adalah spesies tanaman ubat yang sangat jarang berlaku bagi keluarga Zingiberaceae. Ia boleh ditemui di tempat kawasan yang tinggi dan ia adalah endemik kepada Borneo. Ia dipercayai mempunyai sifat perubatan terhadap bakteria yang menyebabkan keracunan makanan. Orang tempatan biasanya menggunakannya untuk menrawat sakit perut, keracunan makanan dan keracunan alkohol. Kajian ini dijalankan untuk menentukan aktiviti antimikrobial B. stenophylla dengan menggunakan kaedah penyebaran agar dan penyekatan minimum penyinaran (MIC) terhadap strain Escherichia coli (E. coli) EDL 993 dan ATCC 25922, strain Pseudomonas aeruginosa (P. aeruginosa) ATCC 27853 dan Bacillus cereus (B. cereus) strain ATCC 33019. Kegiatan antimikrobial yang tinggi telah ditunjukkan terhadap P. aeruginosa dalam kedua-dua kaedah agar penyebaran dan pengurangan konsentrasi minimum, sementara aktiviti antimikrob paling rendah terhadap B. cereus. Sebaliknya, ekstrak yang menggunakan heksana dan etil asetat sebagai sistem pelarut menunjukkan aktiviti antimikrob tinggi berbanding dengan ekstrak menggunakan air sebagai sistem pelarut. Di samping itu, pengekstrakan dari batang menunjukkan lebih banyak aktiviti antimikrob berbanding dengan rizom dan daun. Ekstrak dari B. stenophylla telah menunjukkan aktiviti antimikrob dan kajian lanjut perlu dilakukan dalam vivo pada sifat-sifat perubatan tumbuhan.

***Kata kunci**: B. stenophylla, antimikrobial, agar penyebaran, pemekatan perencatan minimum,kromatografi lapisan nipis*

1.0 Introduction

Some microbes can be harmless while others can cause infection, disease and even death towards human. The harmful microbes are classified as pathogen. The most common illness caused by a pathogen is food poisoning (Doughari *et al.*, 2007; Pirbalouti *et al.*, 2009; Sapkota *et al.*, 2012). Some examples of gram negative bacteria which cause food poisoning are *E. coli* and *P. aeruginosa* while gram positive include *B. cereus* (Solomakos *et al.*, 2008; Pandey and Singh, 2011). Many chemical preservatives has been used to prevent any food poisoning outbreak. However, it can be dangerous as once these microbes is resistance toward the chemical and antibiotics. The emergence of antimicrobial resistant bacteria is occurring rapidly around the world and is threatening the life of patient as the antibiotic is not as effective as before. Antimicrobial resistance is where adequate antimicrobial dosage is no longer able to cure infection or disease which is caused by the pathogen with the resistance gene (Canton & Morosini, 2011). These antibiotic resistance crises occur due to the overuse or misuse of medication, extensive agriculture use and lack of new drug development (Ventola, 2015). According to Davies (2004), evidence shows that the resistance in bacteria can be exacerbated by hypermutation and antibiotic- induced hypermutation. In other words, antibiotic actually acts as a promoter other than being a selector of resistance. Thus, the treatment for the infection and disease became more complicated and new infections will arise which will result in high mortality and higher treatment cost.

In the past, plants have been used widely for medicinal purposes. One of the medicinal plants that can be found in Malaysia is the Zingiberaceace family. The Zingiberaceace family can be used to cure diarrhoea, stomach-ache, dysentery and carminative (Voravuthikunchai, 2007). Plant species which is under the Zingiberaceace

family is the *Boesenbergia* species. *Boesenbergia* is a type of ginger family which can be found in South East Asia. In recent year, researchers have been interested in the *Boesenbergia* species for its antimicrobial properties (Ling et al., 2010). The antimicrobial activity functions to kill or hinder the growth of bacteria, fungi or any microbes which could lead to the development of disease. Countless of studies has been conducted regarding on the antimicrobial activity from different plant extracts as more and more bacteria are resistant toward antibiotic. It has been proven that there is an abundant source of biological active compounds which can be found in the natural extract of medicinal plants which show health benefit properties in studies such as Zainin *et al.* (2013) and Hardi *et al.* (2016). Compared to the drugs which are available today, it proves to be a much safer alternative as it shows less side effects (Sasidharan *et al.,* 2011). Therefore, it is necessary to conduct research on the biological activity of plants and to develop a better understanding on genetic mechanisms of resistance in order to produce new drugs (Nascimento *et al.*, 2000). The aim of this study is to characterize antimicrobial activity of *B. stenophylla* extract against *E. coli, P. aeruginosa* and *B. cereus*.

Objective of studies:

The objective of this study was to determine the antimicrobial activity of *B. stenophylla* extracts against *E. coli, P. aeruginosa* and *B. cereus.*

2.0 Literature Review

2.1 Pathogenic bacteria

One of the criteria for a bacterium to be classified as pathogenic bacteria is that it causes infection and diseases. The bacteria cause infection by invading the host and then multiply within the host cells. Infection and diseases are two different terms in which infection is progression of disease if left untreated. The development of disease depends on the bacteria pathogenicity. Pathogenicity of a bacterium can be measured through the degree of virulence. Death, illness and lesion development can be determined experimentally by measuring the virulence of the bacteria. On the other hand, bacteria can be further categorized into three major groups which are the probable agents for disease, opportunistic pathogen and non-pathogenic. *E. coli, P. aeruginosa* and *B. cereus* are all considered as an opportunistic pathogen and all of them cause food poisoning (Peterson, 1996).

2.1.1 *Escherichia coli*

2.1.1.1 Microbiology of *E. coli*

The most common member of Enterobacteriaceae family is the *E. coli*. It is considered as an opportunistic bacterium and commonly found in the human intestine. All *E. coli* are facultative (Amenu, 2014). Production of acid or acid and gas cause it to ferment glucose and it is also capable of reducing nitrates into nitrites (Brooks *et al.*, 1991).

E. coli is a gram-negative and a rod-shaped bacterium. The length of the bacteria is around 2-4 μm and considers one of the largest bacteria which colonize human. Pili and flagella can be found on the surface of *E. coli*. It is simple to grow as

it only requires a single carbon energy source media. It usually forms convex, circular and smooth colonies with distinct edge on the media. It can be identified with a series of test such as production of indole from tryptophan, Voges-Proskauer reaction and forth more (Brooks *et al.*, 1991).

2.1.1.2 *E. coli* epidemiology

Most of the pathogenic *E. coli* is haemolytic which mean it is capable of destroying red blood cell. The proteins which destroy the red blood cell (RBC) called hemolysin are toxic toward tissue culture and animal experiment. Other than that, the presence of the pili also plays a role in virulence by attaching to the human epithelial surface (Champoux *et al.*, 1990). Serotyping of the O, K and H antigens of the *E. coli* can help distinguish which strains can cause diseases. Diseases which are cause by *E. coli* are urinary tract infection, intestinal infection and meningitis (Davis *et al.*, 1990). The *E. coli* which causes intestinal infection can be further categorized as each strain has different mechanism to cause diarrhoea. The following Table 1 shows the comparison between the classes of *E. coli*.

Table 1: Comparison between classes of *E. coli* that cause diarrhoea

Class of *E. coli*	Clinical syndromes	Epidemiologic Syndromes
Enterotoxigenic (ETEC)	Watery diarrhoea	Infant and traveller in undeveloped country
Enteroinvasive (EIEC)	Dysentery; diarrhoea	usually affect adult; some foodborne outbreak
Enterohemorrhagic	Bloody diarrhoea; Haemorrhagic	one foodborne outbreak

Enteropathogenic (EPEC)	Acute and chronic infant diarrhoea	Outbreak of diarrhoea in infant nurseries

Source taken from Davis *et al.* (1990). Cite right*: Microbiology 4th ed.* Penslyvennia, PA: J. B. Lippincott Company.

2.1.2 *P. aeruginosa*

2.1.2.1 Microbiology of *P. aeruginosa*

P. aeruginosa is a gram negative bacteria under the *pseudomonas* genus or otherwise known as *pseudomonads*. It can be characterized by its rod shape, non-spore forming and the presence of polar flagella and pili feature. It can be further characterized by using pyocyanin and proverdin, a water soluble pigment which can be added with the solid media and will result in a formation of distinctive blue-green colour. In addition, oxidase tests also can be used to test presence of *P. aeruginosa*. It will show a negative result due to the production of indophenol oxidase which render oxidation to occur (Fujitani *et al.*, 2017).

It can be found in a moist environment such as soil, water, fruit and vegetation. It only requires minimal amount of nutrients in order to survive. Nutrients such as acetate and ammonia can be used as a carbon source. *P. aeruginosa* also can grow under anaerobic condition. Due to these features, the bacteria can survive in many different conditions (Fujitani *et al.*, 2017). The range of temperature it require to grow is around 20°C to 42 °C.

P. aeruginosa can cause tissue damage, collagenase and digest elastin by the production of exotoxin A. It commonly causes infections such as otitis externa, infection associated with injuries, pneumonia, keratitis, conjunctivitis, endophthalmitis and cystic fibrosis (Champoux *et al.*, 1990).

2.1.3 B. cereus

2.1.3.1 Microbiology of *B. cereus*

In the bacillaceae family, *B. cereus* is a gram positive bacteria which can live under aerobic to facultative condition. *B. cereus* is characterized as a rod shaped, spore forming bacteria. The spore is protected by layer of coat which makes it less sensitive toward heat, freezing, drying and radiation. These features contribute to its ability to live in vast and harsh environment. It can be widely found in place such as soil, vegetation, decaying organic matter, sediment, marine water and dust. The diameter of the bacteria is around 2 to 7 mm and the optimum temperature for it to grow is around 37 °C (Murray *et al.*, 2007).

B. cereus produces toxin which cause food poisoning. Four hemolysis, three phospholipases and three enterotoxins can be found in the secreted toxin. It causes two types of food poisoning where one result in vomiting while another result in diarrhoea (Granum & Lund, 1997). It also can cause infection such as nongastrointestinal infections, respiratory tract infections, keratitis, nosocomial infection and food poisoning (Bottone, 2010).

2.2 Antimicrobial resistance in bacteria

Production of new drugs is necessary from time to time as more microorganisms are resistant toward drugs. The emergence of antimicrobial resistance affects both gram positive and gram negative bacteria. According to Lowy (2003), it is believed that factors such as improper antimicrobials usage, usage of growth enhancer excessively in animal feed and international and regional travel contribute to the increase of antimicrobial resistance bacteria. The development of resistance gene makes the bacteria capable of adapting to different environment and cause life-threatening infections. The bacteria generally acquire its new ability through de novo mutation or developing the resistance gene from other organism through conjugation, transformation or transduction. The resistance gene mainly stops the

antibiotic action by destroying it through enzyme production or drug target site modification. Moreover, it can stop the drug action through the production of alternative metabolic pathway or inhibit it by expressing efflux system (Tenover, 2006). It is a public health threat as most of the pathogenic bacteria became harder to kill as no available antibiotics are effective. Patients will have to delay their treatments or therapies until there is new treatment discover. Consequently, it will result in high mortality in the hospital as new infection emerge (Nascimento *et al.*, 2000).

2.3 Medicinal plants

In the past, technology was not well developed and not as advanced as it is today. Therefore, there was no artificial medicine, vaccine or antibiotic to cure any illness or diseases. People used to rely on medicinal plant for treatment and it is estimated that around 80% of the people still do rely on it today. According to the Amenu (2014), most of the traditional therapies or medicines are made from natural product such as plant extract. Till this day, natural product such as plant extract is still considered as a major source in studies or research for drugs development (Cos *et al.*, 2006). It has been proven that there is an abundant source of biological active compounds can be found in the natural product of medicinal plants. It proves to be a much safer alternative as it show less side effect compared to an artificial drugs. Most of these phytochemical in the natural product exhibit a health benefit properties such as antidiarrheal, antimicrobial, anticancer, antioxidant and analgesic (Sasidharan *et al.*, 2011). These discoveries have led to a better understanding and act as a basic to drug development. However, only 1% of the 500000 plants species have been investigated on their antimicrobial activities (Palombo, 2011).

2.3.1 Zingiberaceae family

A group of ginger family known as the Zingiberaceace family can be found widely in the Southeast Asia. It is widely used for medical purpose, food, spices, dyes, and perfume etc. Over 53 genera and 1200 species ever recorded under the Zingiberaceace family. Most of the ginger species can be found in India, having a total of 20 genera and 200 species. Each genus has a distinct feature in order to differentiate it. The rhizomes of different species have different colour ranging from pink, pale yellow, greenish blue and forth more. Other than that, some species have scale leave which protect its rhizome while some are absent or presence of leafy shoots such as the *Curcuma* spp. have a pseudostem with clasping leaf sheaths while the *Kaempferia* spp. have a short true stem. The genuses which have been commercially uses in medicinal purpose are *Alpina, Hedychium, Amomum, Elettaria, Kaempferia, Curcuma* and *Zingiber* (Kumar *et al.*, 2013). According to Voravuthikunchai (2007), the most commonly used species which gives benefits to human are *Alpina galangal, Boesenbergia pandurata, Curcuma amada, Curcuma longa, Curcuma zedoaria, Kaempferia galanga, Zingiber officinale* and *Zingiber zerumbet*. Table 2 show the examples of medicinal use in the Zingiberaceace family:

Table 2: Medicinal use of Zingiberaceae family

Species	Medicinal Uses	References
Curcumin longa	rheumatoid arthritis	Lakhan *et al.*, 2015
	Inflammatory bowel disease	
Boesenbergia pandurata	Diarrhoea, Dyspepsia, Wound	Voravuthikunchai, 2007
Alpinia galanga	Flatulence, Vomiting	Kumar et al., 2013
	Stomach-ache	
Kaempferia galanga	Cough, Bronchitis, Asthma	Kumar *et al.*, 2013

2.3.2 *Boesenbergia stenophylla*

The *Boesenbergia* spp. under the (Zingiberaceae) family is native to tropic area such as India and Southeast Asia. In earlier days, Baker used to categorize the ginger under *Kaempferia* genus which later become *Boesenbergia* genus. It is estimated there are 25 species of *Boesenbergia* spp. that can be found in Borneo (Larsen, 2003). It usually can be found in shady places and damp area such as area close to the stream. It has been proven by researcher that there is a health-benefit property within the rhizome part of the *Boesenbergia* spp. (Ling *et al.*, 2010).

Boesenbergia stenophylla, otherwise known as Jerangau merah in Malay is an extremely rare species and is endemic to Borneo. It only can be found at place with high altitude roughly around 3000 ft because it requires low temperature for optimum growth. The medicinal application of *B. stenophylla* helps to treat stomach-ache, food poisoning and alcohol intoxication. It is said that there is a possible threats to the existence of the plant due to factors such as harvesting the plants and climate change (Toyat *et al.*, 2015).

2.3.3 Phytochemical studies of *Boesenbergia stenophylla*

Studies on *Boesenbergia* species phytochemical agent have been conducted throughout the years. Few of the studies were able to characterize the compound in some of the *Boesenbergia* species. The main compound that can be found in the rhizome and the leaf oils of *B. stenophylla* are sesquiterpene which can be further divided into β-selinene, α-elemene, caryophyllene alcohol, γ-muurolene, spathulenol, and kaur-16-ene (Ahmad & Jantan, 2003).

3.0 Materials & Methods

3.1 Materials

The materials utilised in this study were:

Clorox, Distilled water (ddH_2O), 100% ethylene glycol mono-tert butyl ether, 10% ethylene glycol mono-tert butyl ether in distilled water, *E. coli* O157:H7 with strains of EDL933 and ATCC25922, *P. aeruginosa* strain ATCC 27853, *B. cereus* strain ATCC 33019, Nutrient broth (NB), Nutrient agar (NA), crystal violet, 70% ethanol, saffranin, Mueller-Hilton broth (MHB), Mueller-Hilton agar (MHA), sterile cotton swab, microtiter plate, hexane, ethyl acetate, MTT (Thiazolyl blue tetrazolium bromide), Phosphate Buffered Saline (PBS), Sodium Dodecyl Sulfate (SDS), stem hexane extract, stem ethyl acetate extract, stem ddH_2O extract, rhizome hexane extract, rhizome ethyl acetate extract, rhizome ddH_2O extract, leaf hexane extract, leaf ethyl acetate extract, leaf ddH_2O extract and thin layer chromatography (TLC).

3.2 Sample collection

The sample of *B. stenophylla* was taken from Ba' Kelalan, Lawas by Prof Gabriel Tonga from plantation department. After the sample reach UNIMAS, it was immediately wrapped in newspaper and store in 4 °C fridge in Virology Laboratory to keep it fresh for next procedure.

Figure 1: The samples of *B. stenophylla*